

Gibson Assembly

1.

	Recommended Amount of Fragments Used for Assembly		
	2-3 Fragment Assembly	4-6 Fragment Assembly	Positive Control
Total Amount of Fragments	0.02–0.5 pmols* X μ l	0.2–1 pmols* X μ l	10 μ l
Gibson Assembly Master Mix (2X)	10 μ l	10 μ l	10 μ l
Deionized H ₂ O	10-X μ l	10-X μ l	0
Total Volume	20 μ l	20 μ l***	20 μ l

- Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Optimized cloning efficiency is 50-100 ng of vectors with 2-3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps.
- Control reagents are provided for two experiments.
- If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.

2. Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at -20°C for subsequent transformation.

Reference:

New England BioLabs. Gibson Assembly Master Mix- Assembly. Retrieve from <https://www.neb.com/protocols/2012/09/25/gibson-assembly-master-mix-assembly>